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Intragastric Dai-Kenchu-To, a Japanese Herbal Medicine, Stimulates Colonic Motility via Transient Receptor Potential Cation Channel Subfamily V Member 1 in Dogs

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Japanese herbal medicine, also known as Kampo, is used for various diseases in Japan. One of those medicines, Dai-Kenchu-To (DKT), is considered clinically effective for adhesive bowel obstruction and chronic constipation. Although scientific evidence of DKT to improve adhesive bowel obstruction was shown in several previous reports, mechanism of DKT to improve constipation remains unknown. Our aim was to study the effect of intragastric DKT on colonic motility and defecation, and the involvement of various receptors in DKT-induced colonic contractions. Five beagle dogs were instructed with serosal strain-gauge force transducers to measure circular muscle activity at the proximal, middle, and distal colon. Dogs are suitable for a present study to administer the drugs repeatedly to the same individual and look at its effect on colonic motility. We studied the effects of DKT (2.5 or 5 g) administered into the stomach on colonic motility. Muscarinic receptor antagonist atropine, nicotinic receptor antagonist hexamthonium, or 5-hydroxytryptamine-3 receptor antagonist ondansetron was injected intravenously 10 min before DKT administration. Capsazepine, an antagonist to transient receptor potential cation channel subfamily V member 1 (TRPV1), was administered into the stomach 5 min before DKT administration. Intragastric DKT (2.5 or 5 g) induced colonic contractions within 10 min after administration but did not induce defecation. Pretreatment with atropine, hexamthonium, ondansetron, or capsazepine inhibited DKT-induced colonic contractions. These results indicate that orally administered DKT stimulates colonic motility via TRPV1, muscarinic, nicotinic, and 5-hydroxytryptamine-3 receptors, thereby providing scientific support for the efficacy of oral DKT in chronic constipation.

Keywords: colonic motility; constipation; Dai-Kenchu-To; gastrocolonic response; transient receptor potential cation channel subfamily V member 1
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Introduction

Japanese herbal medicine that adapted Chinese traditional medicine has been called Kampo in Japanese. Kampo has many prescriptions, and each prescription consists of several herbal ingredients. A patient is given proper prescriptions according to their symptoms. National medical insurance approved some prescriptions of Japanese herbal medicines about 40 years ago, and more than 100 prescriptions have been approved until now. The approval was based on historical experience that those prescriptions had been used safely for a thousand years, but not based on scientific clinical trials required for an ordinary medicine to be approved (Kanda et al. 2005). Therefore, despite the fact that Japanese herbal medicines have been used for various diseases for these 40 years, the mechanisms of their actions largely remain to be investigated.

Dai-Kenchu-To (DKT), one of these traditional Japanese herbal prescriptions, consists of three active ingredients: dried ginger rhizome, ginseng root, and zanthoxylum fruit. We reported previously that intraluminal administration of DKT into the upper gut evoked phasic contractions at a locally administered site via cholinergic and 5-hydroxytryptamine 3 (5-HT_3) receptors in dogs (Shibata et al. 1999a; Jin et al. 2001). These observations were considered as the scientific support to the clinical effectiveness of DKT for uncomplicated adhesive bowel obstruction and postoperative ileus (Itoh et al. 2002; Nakayama and Tanaka 2009). DKT also appears to be effective for morphine-induced constipation (Sato et al. 2010b) and constipation in children (Iwai et al. 2007) and in parkinsonian patients (Sakakibara et al. 2005), suggest-

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ing that DKT may enhance motor activity of the colon. However, the effects of DKT on colonic motility have not been well studied.

The observation that food intake immediately stimulates colonic motility has been referred to as a 'gastrocolonic response,' and this motor response is considered as an extrinsic neural, probably vago-vagal, reflex (Snape et al. 1979; Shibata et al. 1991). We have shown previously that intragastric administration of capsaicin, the major pungent ingredient of chili, evoked colonic contractions and defecations immediately after the administration in conscious dogs (Shibata et al. 1995). Muscarinic, nicotinic, or 5-HT_3 receptor antagonists inhibited intragastric capsaicin-induced colonic contractions (Shibata et al. 1999b). A capsaicin receptor is the transient receptor potential cation channel subfamily V member 1 (TRPV1) (Immke and Gavva 2006). TRPV1 is a nociceptor for heat and acid and was identified on afferent nerve terminals in enteric neurons of the stomach in humans and rats (Horie et al. 2004; Faussone-Pellegrini et al. 2005). These results suggest that capsaicin may induce a gastrocolonic response via TRPV1, cholinergic, and 5-HT_3 receptors. Among three ingredients of DKT, the dried ginger rhizome and the zanthoxylum fruit have similar pungent tastes compared to chili and are likely to cause similar motor effects to those of capsaicin.

Based on these findings, we hypothesized that intragastric administration of DKT would stimulate colonic motility and defecation via an extrinsic neural reflex involving TRPV1, cholinergic, and 5-HT_3 receptors. The aims of the present study were to investigate, 1) the effect of intragastric administration of DKT on colonic motor activity and defecation, and 2) the involvement of cholinergic, 5-HT_3 and TRPV1 receptors in intragastric DKT-induced colonic contractions.

Materials and Methods

Preparation of animals

Procedures and animal care were performed according to the guidelines of the Animal Care and Use Committee of the Tohoku University. We performed all operative preparations with sterile techniques. Five beagle dogs weighing 10-12 kg (Oriental Yeast Co., Ltd., Tokyo, Japan) were used. Anesthesia was induced by intravenous sodium thiopental (Ravonal; Mitsubishi Tanabe Seiyaku Co., Osaka, Japan) at 20 mg/kg body weight and maintained by inhaled halothane (Fluothane; Takeda Chemicals Co., Osaka, Japan) with oxygen. At the time of operation, we placed a silicone catheter (SH N02; Create Medic Co., Yokohama, Japan) into the superior vena cava via the right jugular vein to administer drugs.

Via a midline laparotomy, four strain-gauge force transducers (F-12IS; Star Medical Inc., Tokyo, Japan) were sewn onto the seromuscular surface of the ileum and colon. A strain gauge force transducer consists of copper-beryllium sheet and attached lead wires 60 cm in length. The copper-beryllium sheet, 0.05 mm in thickness and 12 × 7 mm in size, has four tiny holes at each corner and covered with silicone rubber for the purpose of waterproof (Itoh et al. 1977). Utilizing four holes, strain-gauge force transducers were sutured on the terminal ileum 5 cm proximal to the ileocecal junction, the proximal colon 5 cm distal to the ileocecal junction, the distal colon 10 cm proximal to the peritoneal reflection, and the middle colon with the equal distance between each colonic transducer (Fig. 1). Each transducer was placed with short axis of the copper-beryllium sheet parallel to the long axis of the ileocolon to measure circular muscle contractile activity (Fig. 1). The colonic transducers were termed as C1, C2, and C3, respectively. A silicone catheter to administer the materials was placed in the stomach with its tip at the gastric body. The lead wires from the transducers and silicone catheters were tunneled subcutaneously and drawn out via a stab would between the scapulae. The lead wires and the catheters were covered with a canvas jacket

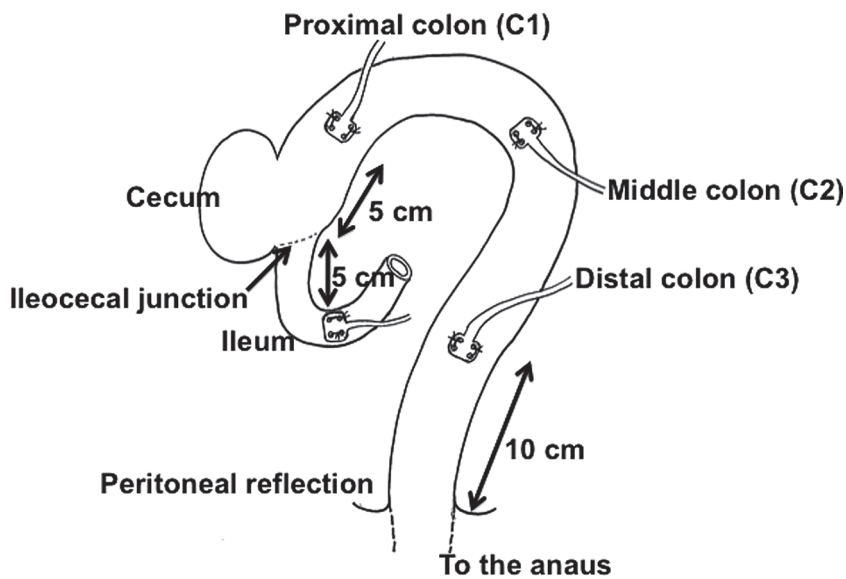

Fig. 1. Schema showing canine preparation.
Four strain-gauge force transducers were sewn onto the seromuscular surface of the terminal ileum 5 cm proximal to the ileocolonic junction, the proximal colon 5 cm distal to the ileocolonic junction (C1), the distal colon 10 cm proximal to the peritoneal reflection (C3), and the middle colon with the equal distance between each colonic transducer (C2).

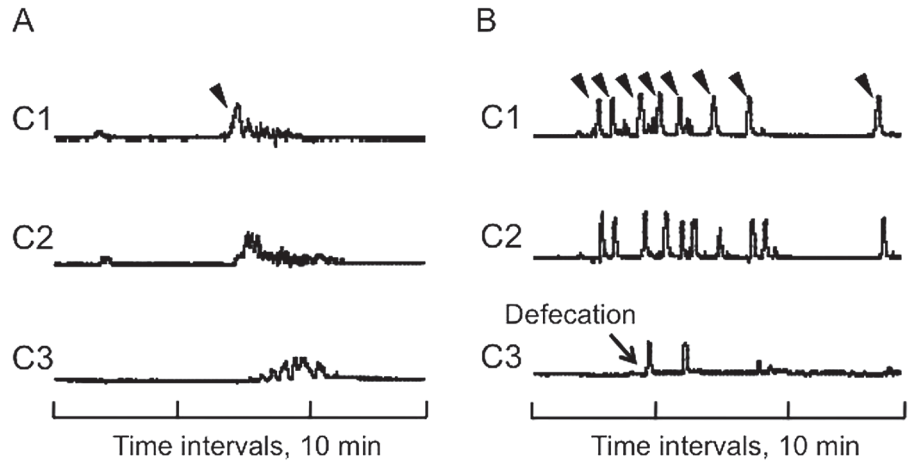

Fig. 2. Representative CMCs and GMCs.
An arrowhead indicates CMCs initiating at the proximal colon C1 (A). Arrowheads indicate GMCs occurring at the proximal colon C1 (B). Defecation was observed, when GMCs reached the distal colon.

for protection from self-inflicted trauma. The dogs were allowed to recover for 14 days after operation.

Colonic motor activity was monitored in the conscious state by connecting the lead wire from the transducers to an amplifier (MS-08; Star Medical), then to a computer, and analyzed by special software (Chart; AD Instruments, Victria, Australia) through Power Lab (AD Instruments). The dogs were fed a mixture of a solid meal (CD-55α; Clea Japan Inc., Tokyo, Japan) and canned food (Vita One Crux; Japan Pet Food Co., Tokyo, Japan) once a day. Water was given ad libitum except for the experimental time.

Two types of colonic contractions were observed in the canine colon as reported previously. One was the colonic motor complex (CMC) consisting of tonic contractions superimposed of phasic contractions, most of which migrated distally with speeds as slow as 4 cm/min (Sarna et al. 1984; Shibata et al. 1993). The other type of contractions was the giant migrating contraction (GMC) which had a very high amplitude and velocity as fast as 60 cm/min. GMCs have been well described and are associated with rapid movement of intracolonic contents, and when GMCs reach the distal colon, defecation occurs (Karaus and Sarna 1987; Shibata et al. 1993). Representative tracings of CMCs and GMCs are shown in Fig. 2.

Experimental protocols

The dogs were fasted for 16 h before each experiment. Seventy mL of 154 mM NaCl solution (Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) containing a powdered extract of DKT (2.5 or 5 g) was administered as a bolus into the gastric lumen through the silicone catheter, when all colonic transducers were in the quiescent period and when ileal contractile activity showed fasted pattern, in order to study the effects of intragastric DKT on colonic motility and defecation. Transducers on the ileum were used to assure that ileal contractile activity exhibited cyclic occurrence of contractions and quiescent period specific to the fasted pattern (Itoh et al. 1978). A powdered extract of DKT consisted of 50% dried ginger rhizome, 30% ginseng root, and 20% zanthoxylum fruit. The colonic motor response induced by 70 mL of 154 mM NaCl solution was used as a vehicle control. DKT was donated by Tsumura and Company, Tokyo, Japan.

To study the mechanism of the colonic motor response induced by intragastric DKT, each of the following antagonists was given intravenously 10 min prior to intragastric administration of DKT (5 g): the muscarinic antagonist atropine (0.1 mg/kg), the nicotinic antagonist hexamethonium (5 mg/kg), and the 5-HT3 receptor antagonist ondansetron (0.5 mg/kg). As a control, 154 mM NaCl (NaCl solution) was given intravenously 10 min prior to intragastric administration of DKT. To study the involvement of TRPV1, the TRPV1 receptor antagonist capsazepine (5 mg) dissolved in 50 mL NaCl solution or 50 mL NaCl solution as vehicle control was administered into the stomach as a bolus 10 min before intragastric administration of DKT (5 g). In order to study the effect of intragastric capsazepine on basal colonic motility, capsazepine (5 mg) dissolved in 50 mL NaCl solution or 50 mL NaCl solution as vehicle control was given into the stomach 10 min before intragastric administration of 70 mL NaCl solution. Atropine, hexamethonium, ondansetron, and capsazepine were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

All experiments were carried out in a random order and repeated twice, and the mean of the 2 studies was regarded as a representative value for that dog. DKT was administrated once a day.

Data analysis

The occurrence of GMCs and the number of defecations within 30 min after intragastric administration of each solution was calculated. The occurrence of CMCs and time lag between intragastric administration of DKT and the occurrence of CMCs were analyzed by visual inspection. The duration and migration velocity of spontaneous and intragastric DKT (5 g)-induced CMCs were also analyzed visually. The area under the contractile waves measured for 30 min after intragastric administration of each solution was used to quantify colonic contractions and expressed as a motility index. We utilized a commercial computer soft ware (Chart; AD Instruments) to calculate the motility index.

All data were expressed as mean ± standard error of the mean (SEM), and P values less than 0.05 were regarded as significant. Analysis of variance (ANOVA) with Scheffe's F test as post hoc was used in analyzing colonic motor response induced by 2.5 or 5 g of DKT. Wilcoxon signed ranks test was used for the comparison between 2 groups.

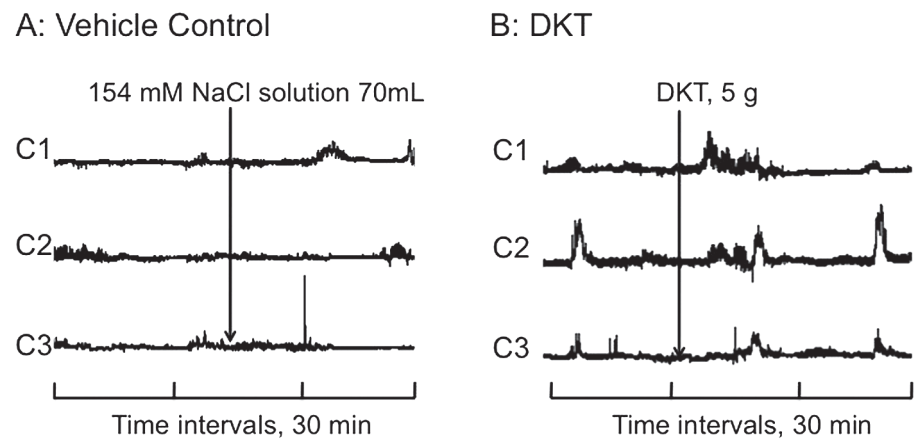

Fig. 3. Effect of intragastric DKT on colonic motility.
Intragastric DKT evoked CMCs in the colon (B), while intragastric administration of 154 mM NaCl solution as vehicle control did not induce any colonic motor response (A).

Table 1. Duration of spontaneous and DKT-induced CMCs.

	C1	C2	C3
Spontaneous CMCs	9.0 ± 1.2	9.2 ± 1.5	11.9 ± 2.2
DKT-induced CMCs	19.0 ± 3.3*	17.2 ± 3.5*	17.0 ± 4.1

The duration of DKT-induced CMCs was greater than that of spontaneous CMCs at C1 and C2 but not at C3. Values are mean ± SEM (min).

Results

Effect of intragastric DKT on colonic motility

Intragastric administration of 70 mL NaCl solution as the vehicle control had no effect on colonic motility (Fig. 3A). Intragastric DKT at doses of 2.5 and 5 g always evoked CMCs within 10 min migrating from C1 to C3 (Fig. 3B). Time lag between intragastric administration of DKT (5 g) and the occurrence of CMCs was 3.4 ± 1.4 min. Intragastric DKT increased the colonic motility index, and the increase was significant at all colonic sites for 5 g and at C2 for 2.5 g (Fig. 4, $p < 0.05$). The duration of these DKT-induced CMCs was greater than that of spontaneous CMCs at C1 and C2 but was not different from spontaneous CMCs at C3 (Table 1). The migration velocity of DKT-induced CMCs did not differ from that of spontaneous CMCs (4.6 ± 1.7 vs. 3.9 ± 2.1 cm/min, $p > 0.05$). Defecations did not occur after intragastric administration of DKT in any dog.

The effect of antagonists on intragastric DKT-induced CMCs

Fig. 5 shows the effects of the different antagonists on the DKT-induced increase in the motility index. Either atropine or hexamethonium prevented the colonic motor response induced by intragastric DKT at all sites ($p < 0.05$). In contrast, ondansetron inhibited in part the intragastric DKT-induced increase in motility index at C1 and C2 ($p < 0.05$) but not at C3. We next analyzed the effect of capsazepine, the TRPV1 receptor antagonist, on the basal motility and the DKT-induced motility. Capsazepine, administered into the stomach 10 min before intragastric administration of 70 mL NaCl solution, had no effect on basal motility (Fig. 6A). However, pretreatment with intragastric capsazepine inhibited colonic motor response induced by DKT at all colonic sites (Fig. 6B and 7, $p < 0.05$ each).

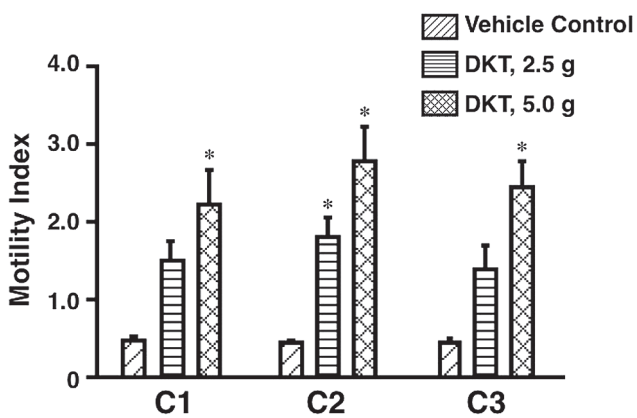

Fig. 4. Motility index in response to intragastric DKT.
DKT increased the motility index at all colonic sites. Values are mean ± SEM, *$p < 0.05$ compared to vehicle control (Scheffe's F test).

Discussion

Recently, complementary and alternative medicine (CAM) has attracted considerable attentions, not only in the public but also in physicians and scientists. CAM is defined

as a group of diverse medical and health care systems, practices, and products that are not considered presently to be part of conventional medicine (Cohen 2003). It has been estimated that about 29% of adults in USA use some form of CAM, with herbal medicines representing the second most common CAM therapy (Ni et al. 2002). These results indicate that herbal medicines are being popular in Western countries. In Japan, DKT is prescribed for the treatment of postoperative adhesive intestinal obstruction and chronic constipation. Crucial concern for the justification of herbal medicine therapies is that more scientific studies addressing their effectiveness and their mechanism of action need to be carried out. With this in mind, our study was designed to explore the mechanisms of action of orally administered DKT on colonic contractile activity.

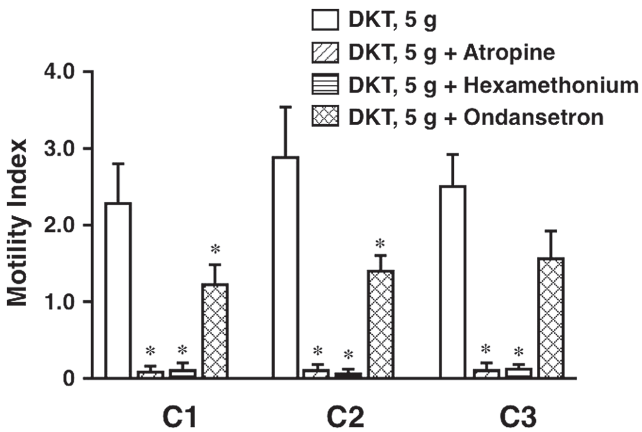

Fig. 5. Effects of specific receptor antagonists on DKT-induced increase of the motility index.
Atropine and hexamethonium prevented the colonic motor response induced by intragastric DKT at all sites, while ondansetron inhibited the increase in motility index at C1 and C2 but not at C3. Values are mean ± SEM, *$p < 0.05$ compared with DKT, 5 g (Wilcoxon signed ranks test).

We demonstrated that intragastric DKT induced CMCs in the colon via TRPV1, cholinergic, and 5-HT_3 receptors. The effects of DKT to stimulate colonic motor activity are not in conflict with previous reports in humans, mice, rats and dogs. In normal healthy volunteers, oral DKT has been shown to accelerate transit through the ascending colonic as measured by scintigraphy (Manabe et al. 2010). In mice, DKT reversed the delay in intestinal transit induced by chlorpromazine in part by cholinergic receptors (Satoh et al. 2003). Similarly in rats, DKT reversed the delay in postoperative intestinal transit via mechanisms mediated by both cholinergic and 5-HT_4 receptors (Fukuda et al. 2006: Tokita et al. 2007). Prior work by Kawasaki et al. (2007) showed that when administered intragastrically, DKT induced burst of colonic contractions in 5 min in conscious dogs. Although atropine or hexamethonium abolished intragastric DKT-induced increase in colonic motility index, ondansetron inhibited it at the proximal and middle but not at the distal colon. The same phenomenon was observed in intracolonic capsaicin-induced colonic contractions (Hayashi et al. 2010).

To the best of our knowledge, this is the first study that shows the involvement of TRPV1 in intragastric DKT-induced colonic contractile activity by using the specific, competitive TRPV1 antagonist capsazepine. (Bevan et al. 1992; Maggi et al. 1993). Tokita et al. (2011) found that inraperitoneal administration of talc caused intraperitoneal

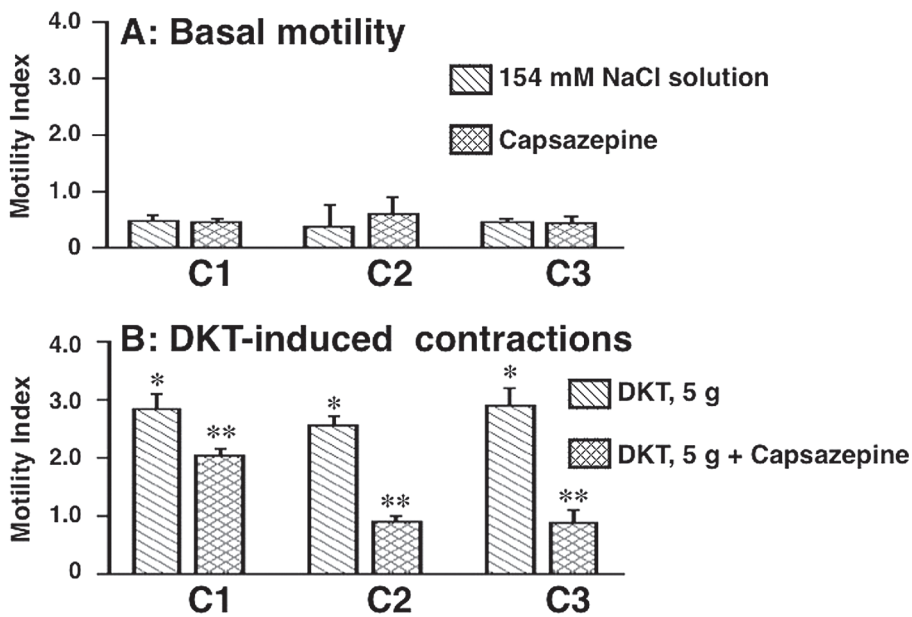

Fig. 6. Effect of capsazepine on basal and DKT-induced colonic contractions.
Capsazepine administered into the stomach did not affect basal colonic motility (A). Capsazepine reduced the increase of colonic motility induced by intragastric administration of 5 g DKT at all sites (B). Values are mean ± SEM, *$p < 0.05$ compared to 154 mM NaCl solution in basal motility, **$p < 0.05$ compared to DKT, 5 g (Wilcoxon signed ranks test).

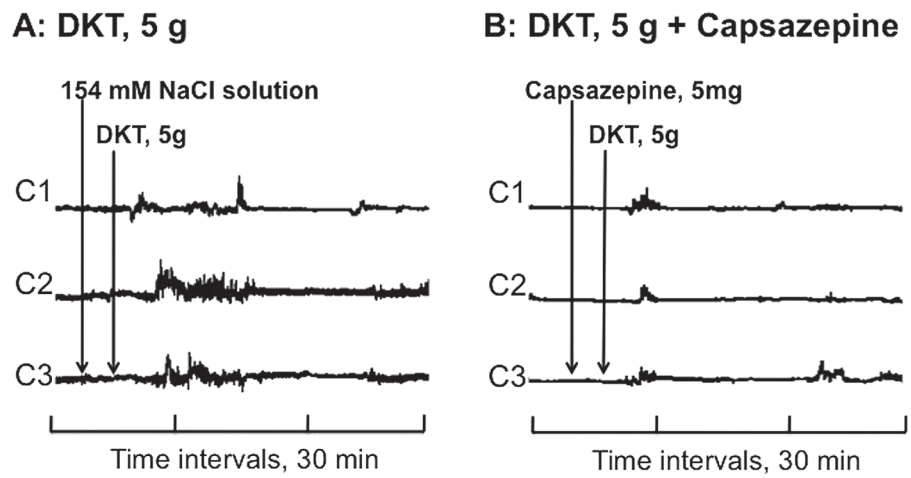

Fig. 7. Effect of capsazepine on colonic contractions induced by intragastric DKT.
Intragastric DKT at the dose of 5 g caused CMCs along the entire colon (A). Intragastric administration of capsazepine prior to administration of intragastric DKT inhibited this colonic motor response (B).

adhesions in rats, and expression of TRPV1 mRNA was increased in the small intestine in rats with adhesions. Subcutaneous injection of capsaicin or intragastric administration of DKT decreased the formation of adhesions induced by intraperitoneal talc. The subcutaneous administration of the non-competitive TRPV1 antagonist ruthenium red inhibited the reversing effect of capsaicin or DKT on adhesion formation, suggesting that the effect of capsaicin or DKT to reverse the talc-induced formation of adhesions was mediated by TRPV1 receptors. These results are consistent with our results in this study, suggesting that some of the actions of DKT are mediated by TRPV1 receptors. Among three ingredients of DKT, the dried ginger rhizome and zanthoxylum fruit are pungent, while ginseng root is not. Because capsaicin is contained in chili and also pungent, substance(s) in the dried ginger rhizome and the zanthoxylum fruit may have caused colonic motor response induced by intragastric DKT.

These observations lend scientific support for the efficacy of oral DKT in the management of chronic constipation by enhancing colonic motility. Because intragastric DKT did not induce defecation within 30 min after the administration, the effect of intragastric DKT does not appear to be as effective as that of intragastric capsaicin, which induced defecation immediately after the administration (Shibata et al. 1995). Oral administration of magnesium citrate and olive oil increased the duration of contractile activity/hour later than 2 hours after the administration and induced defecations with the mean intervals at 7 ± 0.5 hours (Karaus et al. 1987). The number of defecations increased within the first 2 hours and 4-8 hours after the administration of a motilin receptor agonist and sennoside, respectively, in conscious dogs (Hirabayashi et al. 2009). These observations indicate that intragastric DKT may increase the number of defecations later than several hours after the administration. Nevertheless, the effectiveness of DKT on colonic contractile activity shown in the present study supports the observed clinical benefits of DKT on constipation.

We determined the doses of intravenously injected antagonists (atropine, hexamethonium, and ondansetron) according to previous studies in conscious dogs (Mochiki et al. 1997; Haga et al. 1998; Shibata et al. 1995, 1999a, b). There have been no studies in which TRPV1 antagonist capsazepine was administered into the gastric lumen in conscious dogs. The dose of capsazepine in the present study (5 mg) was determined according to a previous report; in that study, right heart injection of capsazepine at the dose of 0.5 mg/kg-min inhibited the lung resistance induced by lactic acid injected into the right heart in newborn dogs (Nault et al. 1999). We consider that the dose of capsazepine in the present study was adequate.

To minimize the number of dogs used in the present study, we reused five dogs several times. Thus, we were able to compare the effects of various reagents in the same animals. In this context, four to six dogs have been used in recent studies from our institution (Hayashi et al. 2010; Sato et al. 2010a) and other institutions (Dong et al. 2005; Morita et al. 2012; Yanai et al. 2013).

One limitation of our study is that we could not determine whether colonic motor response induce by DKT is mediated via an extrinsic neural reflex. Gastrocolonic response induced by feeding and intragastric capsaicin-induced colonic contractions were considered mediated via an extrinsic neural reflex (Shibata et al. 1991, 1995). Intragastric administration of lidocaine prior to intragastric DKT inhibited DKT-induced enhancement of gastrointestinal contractions (Furukawa et al. 1995). We reported previously that DKT administered into the gastrointestinal lumen evoked phasic contractions locally at the sites of administration (Jin et al. 2001). TRPV1 is expressed in nerve endings of primary sensory nerves in the rat stomach (Horie et al. 2004). All these results suggest that intragastric DKT is likely to affect locally, bind to TRPV1 at nerve endings of

primary sensory nerves, and then stimulates colonic motor activity via an extrinsic neural pathway.

In conclusion, intragastric DKT stimulates colonic motor activity via TRPV1, cholinergic, and 5-HT_3 receptors, but did not induce defecations. These observations lend scientific support for the efficacy of oral DKT in chronic constipation.

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Conflict of Interest

C.S. received research support for the study by a Grant from Tsumura and Company, Tokyo. The remaining authors declare no competing financial interests.

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